

Variations in the Anatomical Features of Cotyledon Areoles in Leguminosae

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Variations of anatomical features of the cotyledon areoles in dormant mature seeds were examined in representative species of 23 tribes of subfamily Papilionoideae of the family Leguminosae. The surfaces of the areoles are sunken or flat; the epidermal cells of the areoles are smaller or larger than those of the surrounding cells; the cells of several cell layers abutting inwards against the epidermis of the areoles are usually smaller than those of the surrounding part; stainability of cells of the cotyledon areoles with iron-hematoxylin is usually similar in degree to that of the surrounding cells, but sometimes differs in lacking unstained granules in the cells of the cotyledon areoles.

On the abaxial surfaces and on the midvein of cotyledons in dormant mature seeds of legume species an oval spot with granularly projected epidermal cells was found and named the cotyledon areole (on page 527 in Endo and Ohashi 1997). This areole was found in species which are included in 23 tribes of Papilionoideae (Endo and Ohashi 1998). The substructure of the areole has been studied in *Glycine max* (Miksche 1961), *Melilotus altissima* (Endo and Ohashi 1998) and *Pisum sativum* (Endo and Ohashi 1998). The areoles are composed of granularly projected epidermal cells, hypodermal cells, and several cell layers abutting inwards against the hypodermal cells. These cells are distinguished from the surrounding cells by differences of size and/or stainability with iron-hematoxylin (Endo and Ohashi 1998).

On the other hand, some variations in anatomical features of the cotyledon areoles were observed, i.e., cotyledon areole cells without

epidermis are smaller than the surrounding cells in *Pisum sativum* and *Melilotus altissima* (Endo and Ohashi 1998), but larger in *Glycine max* (Miksche 1961); cotyledon areole cells are more deeply stained by iron-hematoxylin than the surrounding cells in *Melilotus altissima* (Endo and Ohashi 1998) but such differences in stainability were not reported in *Pisum sativum* (Endo and Ohashi 1998) and *Glycine max* (Miksche 1961).

In the present study, we aim to elucidate the variation of anatomical features of cotyledon areoles in the subfamily Papilionoideae of family Leguminosae more precisely.

Materials and Methods

The anatomical structures of cotyledon areoles of seed cotyledons of 25 species, which are classified into 23 tribes in Papilionoideae, were observed. The species examined are listed in Table 1. Sources of the seeds and their voucher specimens are same as shown in Endo

and Ohashi (1998) except *Carmichaelia exsul* and *Glycine max*. The seed source of *C. exsul* is Royal Bot. Gard. Sydney, Australia, and its voucher is Endo 3133 (CBM). The seed source of *G. max* is unknown and its voucher is Endo 3132 (CBM).

To make serial sections of mature seed cotyledons, seeds were softened by soaking in 50% aqueous ethanol for more than one day. For observations of the serial sections by light microscope (LM), materials were fixed with FAA and dehydrated in a t-butyl alcohol series and embedded in "Hist-Paraffin" (m.p. 52–54°C, Wako Pure Chemical Industries, Ltd., Japan). After embedding, the materials were cut into serial sections 4–7 μm in thickness. The sections were stained with a combination of iron-hematoxylin, safranin and fast green FCF. For the scanning electron microscope (SEM), materials were treated according to the standard method.

Results

We observed the following variations in anatomical characters of cotyledon areoles.

1. Surface structure: Surfaces of the cotyledon areoles are flat (Figs. 1, 2) or sunken (Fig. 3).
2. Relative sizes of cells: We compared cell sizes of the cotyledon areole with cells surrounding the cotyledon areole at the epidermis, the hypodermis, and several cell layers abutting inwards against the hypodermis. Differences are as follows:

- (1) Epidermis: the cotyledon areole cells are smaller (Figs. 2–6), or larger (Fig. 7) than or equal to the surrounding cells.

- (2) Hypodermis: the cotyledon areole cells are smaller (Figs. 2–6), or larger (Miksche 1961) than or equal to the surrounding cells.

- (3) Several cell layers abutting inwards against the hypodermis: the cotyledon areole cells are smaller than (Figs. 2–6) or equal to the surrounding cells.

3. The relative stainability with iron-

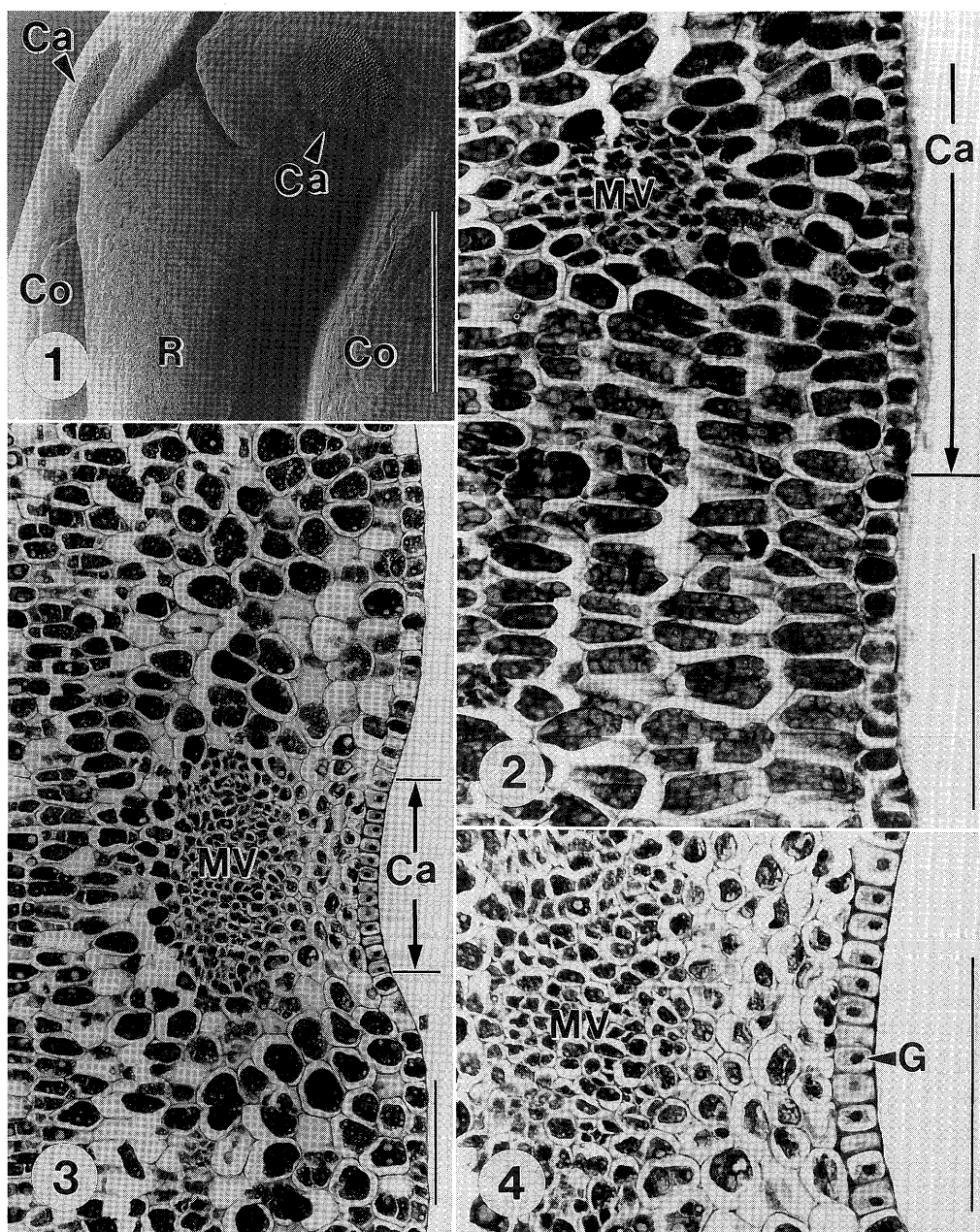
hematoxylin of the two parts, the cotyledon areole and the surrounding part: we observed three types of relative stainability as follows: (1) the cell stainability is similar in degree between the cotyledon areole and the surrounding part, (2) the surrounding cells have many unstained granules but the cotyledon areole cells have none or only a few such granules (Fig. 2), (3) the cotyledon areole cells have one deeply stained granule (c.a. 2.5–10 μm long) but such a granule is obscure in the surrounding cells (Figs. 5–8).

Distribution of these features in the species examined is presented in Table 1.

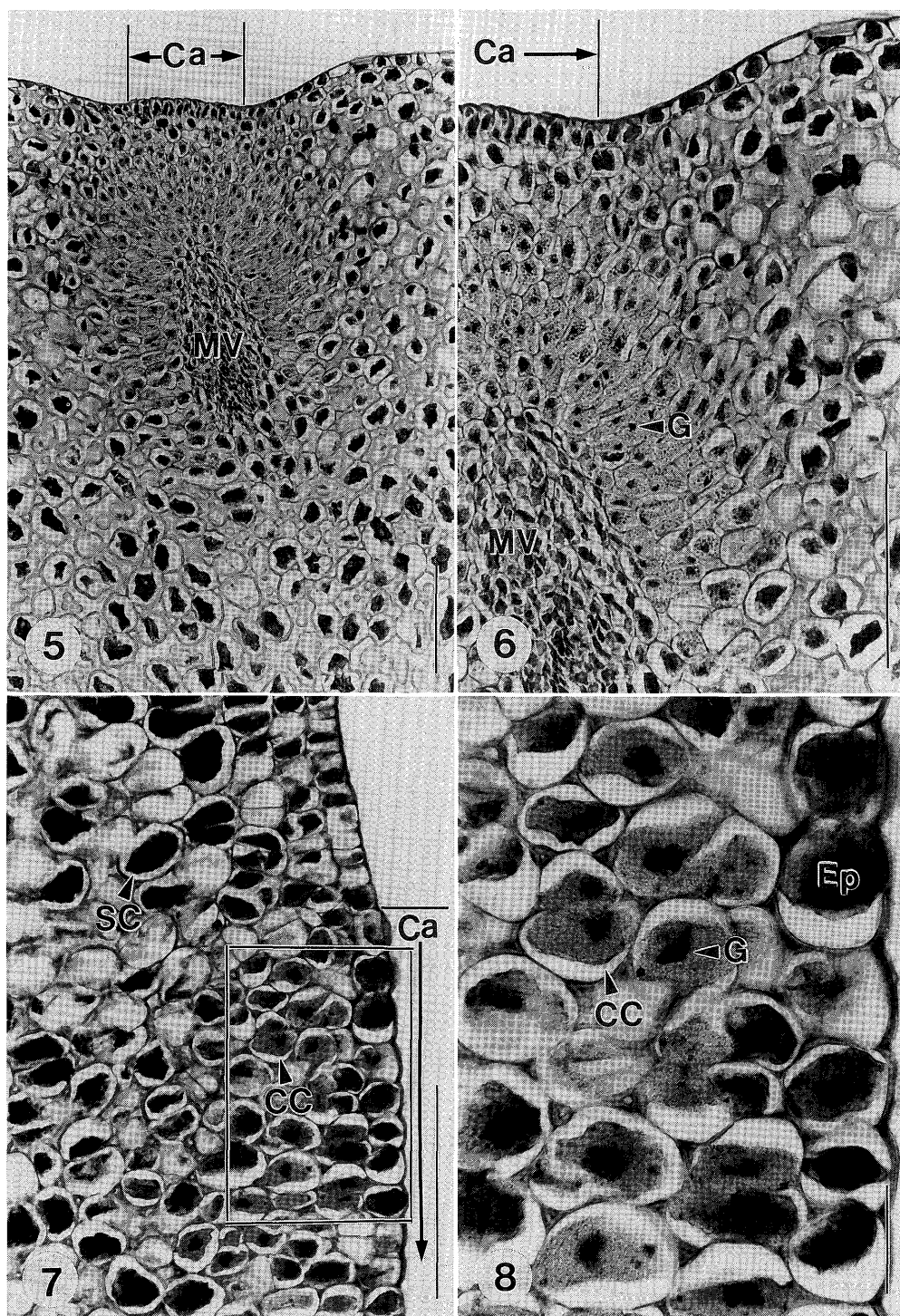
Discussion

In the LM photo of *Pisum sativum* by Endo and Ohashi (1998), the cells surrounding the cotyledon areole have granules unstained with iron-hematoxylin, and the cotyledon areole cells have a few similar but smaller granules (Table 1). The unstained granules of the *P. sativum* cotyledon seem to be starch grains because the shapes of the granules are similar to those of *P. sativum* reported by Bain and Mercer (1966). Therefore, the cells of the cotyledon areole having a few such unstained granules may contain a small amount of such nutrition. This means that the cotyledon areole of *P. sativum* may have a weaker function of storing such nutrition than the surrounding part.

The general features of cotyledon areoles are as follows: the surfaces are sunken or flat; the epidermal cells are smaller or larger than the surrounding cells; the cells in several cell layers abutting inwards against the epidermis of cotyledon areoles are usually smaller than those of the surrounding cells, but rarely only the hypodermal cells of the areoles are larger than those of the surrounding cells; the stainability with iron-hematoxylin is usually similar to that of the surrounding cells, but in several species the cotyledon areole cells are



Figs. 1–4. Surfaces and sections of cotyledons showing the cotyledon areole and surrounding cells. 1. SEM photo of the surface of *Pisum sativum*. 2. LM photo of a section of *Crotalaria* sp. 3, 4. LM photos of a section of *Mirbelia speciosa*. Scale bars = 1 mm in 1 and = 0.1 mm in 2–4. Abbreviations; Ca = cotyledon areole, Co = cotyledon, G = deeply stained granule, MV = primordia of midvein cells, R = radicle.



Figs. 5–8. LM photos of sections of cotyledons showing the cotyledon areole and surrounding cells. 5, 6. *Goodia lotifolia*. 6. Enlargement of the upper right part of Fig. 5. 7, 8. *Anagyris foetida*. 8. Enlargement of the part enclosed with a square in Fig. 7. Scale bars = 0.1 mm in 5–7, and = 0.02 mm in 8. Abbreviations; Ca = cotyledon areole, CC = cotyledon areole cells, Ep = epidermal cells, G = deeply stained granule, SC = the cells surrounding cotyledon areole, MV = primordia of midvein cells.

Table 1. Distribution of anatomical features of cotyledon areoles

Species	Tribe	Features				
		1	2	3	4	5
<i>Glycine max</i> (L.) Merr.	Phaseoleae	l	l	s	a	su
<i>Abrus precatorius</i> L.	Abreae	l	e	e	a	fl
<i>Hedysarum austrosibiricum</i> B.Fedtsch.	Hedysareae	l	e	e	a	fl
<i>Cicer arietinum</i> L.	Cicereae	l	e	s	b	fl
<i>Carmichaelia australis</i> R.Br.	Carmichaelieae	l	s	s	a	fl
<i>Glycyrrhiza pallidiflora</i> Maxim.	Galegeae	l	s	s	a	fl
<i>Carmichaelia exsul</i> F.Muell.	Carmichaelieae	l	s	s	a	su
<i>Podalyria sericea</i> R.Br.	Podalyrieae	l	s	s	a	su
<i>Tadehagi triquetrum</i> (L.) H.Ohashi ssp. <i>pseudotriquetrum</i> (DC.) H.Ohashi	Desmodieae	l	s	s	a	su
<i>Melilotus altissima</i> Thuill. **	Trifolieae	l	s	s	b	fl
<i>Trigonella foenum-graecum</i> L.	Trifolieae	l	s	s	b	fl
<i>Pisum sativum</i> L. **	Vicieae	l	s	s	b	fl
<i>Tephrosia purpurea</i> (L.) Pers.	Tephrosieae	e	e	e	a	su
<i>Psoralea bituminosa</i> L.	Psoraleae	s	s	e	a	fl
<i>Coronilla emerus</i> L.	Coronilleae	s	s	s	a	fl
<i>Nissolia fruticosa</i> Jacq.	Aeschynomeneae	s	s	s	a	fl
<i>Indigofera zollingeriana</i> Miq.	Indigoferae	s	s	s	a	su
<i>Petteria ramentacea</i> (Sieber) C.Presl.	Robinieae	s	s	s	a	su
<i>Sesbania cannabina</i> (Retz.) Poir.	Sesbanieae	s	s	s	a	su
<i>Tetragonolobus palaestinus</i> Boiss. & Bl.	Loteae	s	s	s	a	su
<i>Crotalaria</i> sp.	Crotalarieae	s	s	s	b	fl
<i>Cytisus scoparius</i> (L.) Link	Genisteae	s	s	s	b	su
<i>Anagyris foetida</i> L.	Thermopsidaeae	s	s	s	c	su
<i>Goodia lotifolia</i> Salisb.	Bossiaeeae	s	s	s	c	su
<i>Mirbelia speciosa</i> Siebold	Mirbelieae	s	s	s	c*	su

Features: 1, relative size of epidermal cells of cotyledon areoles and those of the surrounding parts; 2, relative size of hypodermal cells at cotyledon areoles and those at the surrounding parts; 3, relative size of the cells of several cell layers abutting inwards against hypodermis at cotyledon areoles and those at the surrounding parts; 4, stainability of cotyledon areole cells in comparison with the surrounding cells; 5, surface structure.

Abbreviations: a = cell stainability is similar in degree between the cotyledon areole cells and the surrounding cells; b = the surrounding cells have many unstained granules but the cotyledon areole cells have none or only a few such granules; c = the cotyledon areole cells have one deeply stained granule (c.a. 2.5–10 μ m long) but such a granule is obscure in the surrounding cells; c* = the epidermal cells of the cotyledon areole have one deeply stained granule but such a granule is obscure in those cells of the surrounding part (Figs. 3, 4); e = the cotyledon areole cells are almost equal to the surrounding cells in size; fl = flat; l = the cotyledon areole cells are larger than the surrounding cells; s = the cotyledon areole cells are smaller than the surrounding cells; su = sunken; ** = data from photos by Endo and Ohashi (1998).

distinguished from the surrounding cells by loss of unstained granules in the cells.

Cotyledon areoles were thought to be an apomorphy in Papilionoideae on the bases of outgroup comparison (Endo and Ohashi 1998). The cotyledon areoles share the following

features: 1) appearing at the abaxial side of cotyledons; 2) appearing just on the midvein of cotyledons; 3) the epidermal cells of cotyledon areoles projecting granularly. Therefore, the cotyledon areoles are thought to have a monophyletic origin and are thought to have

an original function in the Papilionoideae. On the other hand, cotyledon areoles have the variations in anatomical features shown in Table 1. Are there any functional variations in the cotyledon areoles? We need further investigation to resolve this problem.

To evaluate taxonomical meanings of the features of cotyledon areoles discovered in the present study, we need further study on stability of these features.

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遠藤泰彦^a, 大橋広好^b: マメ科植物の cotyledon areole の解剖学的特徴の変異

マメ科植物の、成熟した休眠中の種子中の子葉には、ドーム状に隆起した表皮細胞が集まり、楕円形の小区画を成している状態が認められる。この構造は子葉の背軸面で、かつ中央脈の直上に位置している。これを cotyledon areole と命名した (Endo and Ohashi 1997, p. 527)。これまでにマメ科マメ亜科植物の23連から報告されている (Endo and Ohashi 1998)。しかし、その機能は不明であり、分類学的意義に関する研究も端緒についたばかりである。本研究では、同構造の存在が認められた23連から各1 (または2) 種を選択し、cotyledon areole の解剖学的特徴の変異を詳細に調べた。(1) cotyledon areole 構成細胞は周囲細胞とサイズが異

なる。すなわち、表皮の細胞は、より大きいか、あるいはより小さい。また、下皮と主脈へ向かう数層の cotyledon areole 細胞は、通常、その周囲の細胞に比べ小型となるが、稀に、下皮細胞のみがより大型となる。(2) cotyledon areole 細胞の染色性は多くの場合周囲の細胞と異ならない。しかし、鉄ヘマトキシリンで染色されない顆粒を cotyledon areole 細胞にほとんど含まない数種では cotyledon areole 細胞は濃く染色されることにより周囲の細胞からはっきりと識別できた。

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